Remarks

Claims 1-16 are canceled, claims 17-24 are pending. Claims 17 and 19-22 are under examination; Claims 18 and 23 are withdrawn from consideration. New claim 24 is added.

The Office Action states that newly submitted Claim 23 is directed to an invention that is independent or distinct from the invention originally claimed. It is further stated the invention of Claim 23 has different steps and produces a materially different effect from the invention of Claims 17-22.

Applicants respectfully traverse the rejection, and request rejoinder of Claims 17, 19-22 and 23, and submit that the claims are drawn to a common invention, where a candidate compound is analyzed for a biological activity of interest (Claim 17) or characterized according to its biological activity (Claim 23). Both claims relate to a process wherein a candidate agent is contacted with cells in culture stimulated by a "plurality", or "by at least two", factors. The methods of both Claims 17 and 23 record changes in cellular parameters to generate a "biomap", or "biological data set profile".

Applicants further note that co-pending patent filings by Applicants have been amended during prosecution to contain claim language similar to that of Claim 23, for example recently issued U.S. Patent no. 7,266,458 recites:

1. A method for characterization of a genetic agent according to its mechanism of action on cellular signaling pathways, the method comprising:

expressing said genetic agent in a cell, wherein said cell is present in a cell culture assay combination, wherein said cell culture assay combination comprises cells and at least two factors sufficient to provide a physiological state of interest involving at least two pathways;

recording changes in at least two different cellular parameter readouts whose levels vary as a result of introduction of said genetic agent;

deriving a biomap dataset from said parameter readouts wherein said biomap comprises data normalized to be a ratio of test to control data on the same cell type under control conditions in the absence of said genetic agent, and said parameters are optimized so that the set of data in the biomap is sufficiently informative that it can discriminate the mechanism of action of said agent; and

analyzing said biomap by a multiparameter pattern recognition algorithm to quantify relatedness of said biomap to reference biomaps that include known genetic agents that target specific pathways, wherein the presence or absence of relatedness to said reference biomaps provides a characterization of said genetic agent mechanism of action.

In view of the above remarks, rejoinder is requested.

Claims 17 and 19-22 have been rejected under 35 U.S.C. 102(e) as anticipated by Friend *et al.*, U.S. Patent no. 6,801,859 as evidenced by Cole et al., U.S. Patent no. 5,342,777.

Applicants respectfully submit that the presently claimed invention is not anticipated by the cited reference. The invention of the present claims, as set forth in independent Claim 17 and Claim 23, recites the step of contacting a mammalian cell culture with a compound to be characterized, where the cells in the culture are activated by at least two factors. The cited reference describes methods for screening molecules using cell co-cultures, wherein the cell cultures differ by expression of a single target gene by over- or under-expression. The methods do not employ a cell culture comprising at least two factors or in which a plurality of pathways are activated.

The Office Action states that it is inherent to the culture of mammalian cells to include a plurality of factors that affect a plurality of signaling pathways as evidenced by Cole *et al.*, who demonstrate the culture of mammalian liver cells in a culture medium having growth promoting amounts of factors such as epidermal growth factor and retinoic acid.

Applicants respectfully submit that the cited secondary reference may teach that cells can be grown in medium comprising multiple factors. However, the reference does not teach the importance of screening biological agents in cell cultures stimulated in a plurality of signaling pathways.

In the methods of the present invention, a test agent contacts cells in culture that are stimulated in multiple pathways by the addition of at least two factors. Applicants respectfully submit that there is no teaching by Friend *et al.* in combination with Cole *et al.* that would inform one of skill in the art to perform such analysis in the presence of at least two factors acting on the cell.

Applicants have observed that the activation of cells in multiple pathways reveals properties of test agents that are cryptic in the absence of these factors. Many biologically active agents were found to have no detectable change in parameters when brought into contact with unstimulated cells. Yet when added to cells stimulated in multiple pathways, as in the methods of the invention, distinctive parameter changes could be observed.

For example, as shown in Table 1, the compounds AA861, SB 203580, PD098059, and AG126 can be added to a culture of unstimulated HUVEC cells, and after 24 hours the parameter read-outs for ICAM-1, VCAM-1, E-selectin, IL-8, CD31, HLA-DR and MIG would be indistinguishable from controls lacking the agents; and thus would be indistinguishable from each other. Yet, when applied to stimulated cells each of these agents generated a distinctive profile typical of the mechanism of action, *i.e.* NFκB signaling pathway vs. p38 MAPK pathway vs. P42/44 MAPK signaling pathway, *etc.*

The Office Action asserts that Friend *et al.* teaches the use of mammalian cell cultures, at column 44, line 39-40 and column 10 lines 56-59, in the context of cell systems having perturbed biochemical pathways. Applicants respectfully submit that the Examiner's citations are not understood.

Col. 10, lines 56-59 read as follows:

> In most preferred embodiments of the invention, the cells used for cluster analysis are of the same type and from the same species as the species of interest. For example, human kidney cells are preferably tested to identify consensus profiles to evaluate drugs or therapies that are used to treat disorders involving human kidney cells. However, in some preferred embodiments, the biological samples are not of the same type or are not from the same species as the species of interest. For example, in certain preferred embodiments, yeast cells may be used to define consensus profiles that are useful, e.g., in comparing or evaluating drugs or drug candidates used or intended for human therapies.

Col. 44, lines 39-40 read as follows:

> For each of the mammalian expression systems described above, as is widely known to those of skill in the art, the gene of interest is put under the control of the controllable promoter, and a plasmid harboring this construct along with an antibiotic resistance gene is transfected into cultured mammalian cells. In general, the plasmid DNA integrates into the genome, and drug resistant colonies are selected and screened for appropriate expression of the regulated gene. Alternatively, the regulated gene can be inserted into an episomal plasmid such as pCEP4 (Invitrogen, Inc.), which contains components of the Epstein-Barr virus necessary for plasmid replication.

Applicants respectfully submit that the cited sections of Friend *et al.* do not teach a method of analysis wherein an agent contacts a mammalian cell culture, wherein said culture comprises a plurality of factors and wherein a plurality of pathways are induced by the presence of the factors. The cited paragraph from column 10 suggests that screening drugs for treatment of kidney cancer might use kidney cancer cells, but that in preferred embodiments yeast cells are used. The cited paragraph from column 44 teaches a gene of interest may be expressed on a vector. It is not seen how these sections, when properly read in their entirety, could be interpreted as teaching cell cultures activated by multiple factors.

Applicants further note newly added Claim 24, which specifically recites the addition of factors at the same time as the candidate agent is added to the culture. As discussed above,

Applicants do not believe the Friend et al. teaches the addition of factors in a screening assay. The Cole reference refers to factors that are constitutive to the cell culture – these are not added exogenously at the time of the test agents. An embodiment of Applicants' invention is the approach of adding the factors for a limited period of time that is carefully controlled, for example at the same time as the test agents, as set forth in newly added Claim 24, or at a nearby time that is consistent. This approach provides a particularly desirable reproducible system for analysis of the biological effect of an agent.

Applicants further note that Friend *et al.* fails to teach specific aspects of the invention recited in Claim 23. Claim 23 recites a number of features important to the success of the claimed methods, including the use of at least two factors; the measurement of at least three different parameters; the ratio normalization of parameter data against control data to obtain a biomap, and multiparameter pattern recognition as a means to quantify the relatedness of a test biomap to reference biomaps, to characterize the mechanism of action of a test agent.

Applicants respectfully submit that the combined use of the factors, parameters, multiparameter pattern recognition and reference biomaps provides for unexpected benefits not found in the prior art, in that the methods of the invention provide an analysis of sufficient discrimination that the mechanism of action of a test compound can be determined. Such discrimination cannot be obtained using the methods set forth in the cited prior art.

The methods of the invention are based on the synergistic effect of specific features in the claimed invention. Features in the instant claims that are important to the practice of the methods include:

- **Cells with activated pathways**. In the methods of the invention, cells are stimulated in a plurality of pathways by the addition of a plurality, or of at least two factors.
- **Normalization of results**. The results obtained with an agent are normalized to results obtained with the activated cells in the absence of the agent.
- **Reference Biomaps**. The parameter data in biomaps is classified by comparison with reference biomaps.
- **Mechanism of action**. The methods of the invention provide sufficient information that a test compound can be classified according to its mechanism of action.

Applicants respectfully submit that the unexpected benefits of the claimed invention are evident in the Examples provided in the present application.

The experiments set forth in paragraphs 183-186; Figures 4A – 4C; and Table 1 are illustrative of the unexpected benefits of Applicants' methods, and provide a useful comparison with the cited prior art.

Exemplary parameter data for a large number of agents are shown in Table 1, and the mechanism of action information obtained with multiparameter clustering analysis are shown in Figure 4C.

Some selected data sets from Table 1 include:

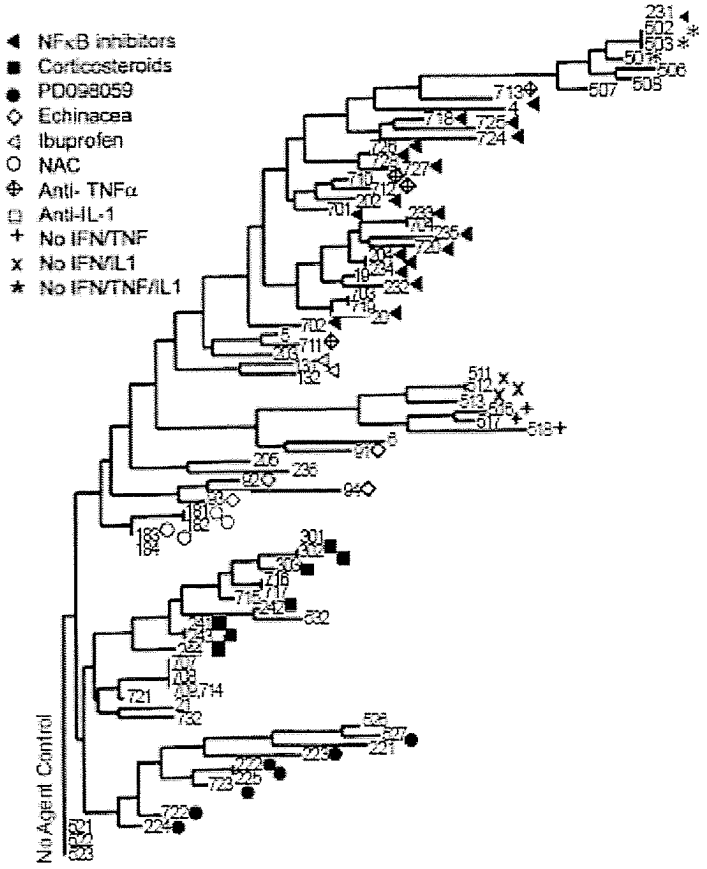
Inhibitor Class	UID	Compound	Conc.	Units	1	2	3	4	5	6	7
Antioxidant	181	N-acetylcysteine	5.00	□M							
Corticosteroid	241	Prednisolone	160.00	□M							
NF□B	4	AA861	20.00	□M					ND		
p38 MAPK	730	SB 203580	80.00	□M						ND	
p42/44 MAPK	221	PD098059	18.70	□M					ND	ND	
Tyr Kinase	733	AG126	25.00	□M							
N/A	521	Control									

Applicants wish to point out what is evident from even this small subset of Table 1 information – that no single parameter can sufficiently characterize the mechanism of action for an agent. If one views the information provided in Table 1 as a single parameter point, next to another single parameter point, one could never determine the pathways in which a test agent is acting. It is only when one considers multiple parameter readouts simultaneously, and in normalized datasets, that one can distinguish the mechanism of action.

Applicants note that the cited prior art does not teach methods wherein an agent is contacted with a cell culture in the presence of factors stimulating a plurality of pathways. Applicants have observed that the activation of cells in multiple pathways reveals properties of agents that are cryptic in the absence of these factors. Many biologically active agents were found to have no detectable change in parameters when brought into contact with unstimulated cells. Yet when added to cells stimulated in multiple pathways, as in the methods of the invention, distinctive parameter changes could be observed.

The clustering schematic of Figure 4C represents the result of multiparameter analysis of this data, where multiple parameter readouts are simultaneously evaluated for a test agent, which groups these compounds according to their mechanism of action, as follows:

FIG. 4C



These data demonstrate that the response of primary cells to agents in a complex environment containing multiple exogenous factors are sensitive and reproducible enough for utilization as a screening method to identify the mechanism of action of a test agent.

In comparison, the Examiner's attention is drawn to the methods of Friend *et al.* Friend *et al.* teach methods that would not have suggested those of the invention to one of ordinary skill in the art at the time the present invention was made. The Friend *et al.* methods are characterized as follows:

> Specifically, these methods allow screening for modulators of biomolecules, including screening for antimicrobial agents, by the use of cell types which exhibit conditional growth phenotypes. . . .
>
> The method includes determining the effects of a potential modulator by comparing a phenotypic sensor between a first cell type having an altered biomolecule and a second cell type having a normal biomolecule. The cell type having an altered biomolecule has a conditional growth phenotype, or the altered biomolecule has a partially crippled function. . . . At least one of the first and second cell types are grown in contact with a potential modulator in an appropriate growth medium, preferably under semi-permissive conditions, such that the

> function of the altered biomolecule is partially crippled. Preferably the first and second cell types are separately in contact with the potential modulator. . . .
>
> A "modulator" or 'modulator of a biomolecule" or "biomodulator" is an agent which is able to affect the activity of a biomolecule by either inhibiting or enhancing that activity. Generally, such a modulator is an inhibitor of the biomolecule. Thus, modulators include, for example, antimicrobial agents and anticancer agents. A "known biomodulator" is a compound which is known to be biologically active on particular cell, but the particular mode of action or cellular target need not be known. A "potential modulator" is a test compound is a screen or evaluation.

The methods taught by Friend *et al.* differ from those of the present invention in important ways that highlight important aspects of the invention. Friend *et al.* methods do **not**:

- Stimulate specific multiple pathways
- Normalize results against activated cultures in the absence of the agent.
- Involve mechanism of action determination and reference biomaps. Friend *et al.* methods do not involve the performance of such an analysis.

The present claims are drawn to various methods for generating and utilizing a dataset of parameter values obtained from cells under specific culture conditions – termed a "biomap" or a "biomap profile" – in determining the effect of a genetic agent on a cellular signaling pathway. The subject methods provide robust results having enhanced predictability in relation to a physiological state of interest, by providing for the culture systems where multiple pathways are induced, and where multiple parameters are measured and compared to control assay combinations.

Applicants' invention provides methods that harness cellular complexity to provide insight into the pathways affected by a genetic agent of interest. Such analysis provides a much deeper understanding of gene action than can be readily obtained by methods taught by the prior art, and such a deeper understanding is very useful in drug development, elucidation of cellular signaling pathways, and the like.

Biological responses, particularly responses in primary human cells, can display significant variability from day to day and from donor to donor. One important aspect of the present invention is that, while the levels of determined parameters can vary substantially between assays, combinatorial responses involving multiple pathways are less variable. Thus, the process of normalization used to produce a biomap provides cellular activity profiles that are robust and reproducible.

In contrast, Friend et al., which primarily rely on a simplified cell model provided by yeast cells, find that:

> The methods of the present invention include: (i) obtaining or providing response profiles for the biological response (or responses) of interest; (ii) defining sets of co-regulated cellular constituents (i.e., genesets) in the response profiles; and (iii) identifying common response motifs among the defined sets of co-regulated cellular constituents which are associated with particular biological responses such as drug effectiveness or toxicity. The common response motifs thereby identified comprise the consensus profiles of the invention. In preferred embodiments, the methods of the invention further include the step (iv) of "projecting" the original response profiles onto the genesets identified in step (ii) above. Simplified, reduced-dimension response profiles are thereby produced which are more simply and robustly related to biological properties such as drug effectiveness and toxicity.

In contrast, Applicants' invention does not define sets of co-regulated cellular constituents (step ii above) or step (iii) above, defining common response motifs among the sets. Friend et al. require these steps because the reference is directed to deriving a consensus profile from a desired or "ideal" agent (sifting through all of the transcripts to find a set that reproducibly co-regulates). In the case of Friend *et al.*, for every "ideal" agent that they would like to compare against, they will derive a different consensus profile. Thus, every different consensus profile will contain a different gene set. In the present invention, the same cellular constituents (i.e. readout parameters) are determined for all test agents, and cellular constituents that are not altered by the "ideal" agent are just as important as those that are up or down-regulated. Indeed, Friend et al. emphasizes the use of co-varying cellular constituents, whereas the present invention selects cellular constituents that preferably do not co-vary, and are independent.

Applicants respectfully submit that the cited art fails to teach every element of the claimed invention. In view of the above remarks, withdrawal of the rejection under 35 U.S.C. 102 is requested.

Claims 17 and 19-22 have been rejected under the ground of nonstatutory obviousness-type double patenting as being unpatentable over Claims 1-16 of U.S. Patent no. 6,656,695. Applicants have enclosed herewith a terminal disclaimer. Withdrawal of the rejection is requested.

Claims 17 and 19-21 have been provisionally rejected on the ground of nonstatutory obviousness type double patenting as being unpatentable over Claims 1, 7, 9, 10, 14, 33, 34 and 35 of co-pending application no. 10/220,999.

Applicants respectfully submit, as set forth in MPEP 804,

> The "provisional" double patenting rejection should continue to be made by the examiner in each application as long as there are conflicting claims in more than one application unless that "provisional" double patenting rejection is the only rejection remaining in one of the applications. If the "provisional" double patenting rejection in one application is the only rejection remaining in that application, the examiner should then withdraw that rejection and permit the application to issue as a patent, thereby converting the "provisional" double patenting rejection in the other application(s) into a double patenting rejection at the time the one application issues as a patent.
> If the "provisional" double patenting rejections in both applications are the only rejections remaining in those applications, the examiner should then withdraw that rejection in one of the applications (e.g., the application with the earlier filing date) and permit the application to issue as a patent. The examiner should maintain the double patenting rejection in the other application as a "provisional" double patenting rejection which will be converted into a double patenting rejection when the one application issues as a patent.

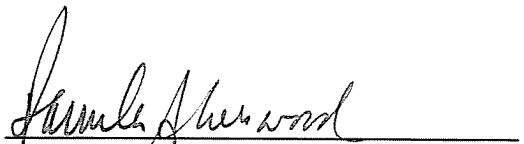
Therefore, it is proper to allow the present application to issue, thereby converting the provisional rejection to a double patenting rejection.

CONCLUSION

The Commissioner is hereby authorized to charge any other fees under 37 C.F.R. §§ 1.16 and 1.17 which may be required by this paper, or to credit any overpayment, to Deposit Account No. 50-0815, order number SEEK-001CON.

Respectfully submitted,

Date: October 26, 2007

By: 
Pamela J. Sherwood, Ph.D.
Registration No. 36,677

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, CA 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231